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transition from the active state to the intermediate state. In the absence of calcium the rapid decrease in fluorescence was followed by a slower fluorescence increase as the inactive state became populated. Acrylodan labeled smooth muscle tropomyosin behaved much like labeled skeletal muscle tropomyosin (Borrego-Diaz & Chalovich, 2010). When the same experiment was done with acrylodan labeled tropomyosin-actin-caldesmon a similar pattern of fluorescence decrease and increase occurred. The rapid decrease corresponded to movement to the intermediate state and could not be distinguished from the rate of S1-ATP detachment. The slower redevelopment of fluorescence differed from the case with troponin in that it occurred as caldesmon binding increased to occupy sites vacated by S1. Changes in caldesmon binding were monitored by NBD probes on caldesmon. We sometimes observed an increase in acrylodan fluorescence when caldesmon was mixed with acrylodan-tropomyosin alone. Thus in the case of caldesmon, S1-ATP detachment occurs with a rapid fluorescence decrease that was independent of caldesmon. The slow fluorescence redevelopment occurred as caldesmon bound to actin-tropomyosin. We have not determined if in that case the fluorescence change coincided with tropomyosin movement.

618-Pos Board B418
Cardiac Troponin T: A Sarcomeric AKAP, Tethers Protein Kinase a at the Myofilaments
Amelia Sumandea, Mary Garcia-Cazarin, Catherine Bozio, Gail Sievert, C. William Balke, Marius P. Sumandea.
Efficient and specific phosphorylation of PKA substrates, elicited in response to beta-adrenergic stimulation, require spatially confined pools of PKA anchored in proximity of its substrates. PKA-dependent phosphorylation of cardiac sarcomeric proteins has been the subject of intense investigations. Yet, the identity, composition, and function of PKA complexes at the sarcomeres have remained elusive. Here we report the identification and characterization of a novel sarcomeric AKAP (A kinase anchoring protein), cardiac troponin T (cTnT). Using the yeast two-hybrid technology in screening two adult human heart cDNA libraries, we identified the regulatory subunit of PKA as interacting with human cTnT bait. Immunoprecipitation studies show that cTnT is a dual specificity AKAP, interacting with both PKA-regulatory subunits type I and II. The disruptor peptide Ht31, but not Ht31P (control), abolished cTnT/PKA-R association. Truncations and point mutations identified an amphipathic helix domain in cTnT as the PKA binding site. This was confirmed by a peptide SPOT assay in the presence of Ht31 or Ht31P (control). Gelsolin-dependent removal of thin filament proteins also reduced myofilament-bound PKA-type II. Using a cardiac troponin (cTn) exchange procedure that substitutes the endogenous cTn complex with a recombinant cTn complex we show PKA-type II is troponin-bound in the myofilament lattice. Displacement of PKA-cTnT complexes correlates with a significant decrease in myofibrillar PKA activity. Taken together, our data propose a novel role for cTnT as a dual-specificity sarcomeric AKAP.

619-Pos Board B419
Effect of Hypertrophic Cardiomyopathy Mutations on Protein-Protein Interactions in the Thin Filament
Shannamar Dewey, Qian Xu, Susan Nguyen, Aldrin V. Gomes.
Familial hypertrophic cardiomyopathy (FHC) refers to a heterogeneous group of cardiac diseases linked to over 630 mutations in sarcomeric proteins. Analysis of the phenotype of the associated disease, the disease outcome, and the functional consequences of FHC mutations all indicate a complex process of pathogenesis that cannot be predicted solely by the localization of the mutation. However, mutations linked to HCM patients tend to increase the calcium sensitivity of contraction in skinned fiber and myosin ATPase experiments. Mutations linked to dilated cardiomyopathy (DCM), however, are commonly associated with a decrease in calcium sensitivity of contraction. These generalizations do not hold true for all FHC mutations. The process of myocardial contraction and relaxation also depends on complex protein-protein interactions within the sarcomeric unit. Altered myocardial contractility resulting from disturbed interaction profiles of sarcomeric proteins may play a critical role in the pathogenesis of FHC. A few studies that have investigated protein-protein interactions involving one protein with a cardiomyopathy-linked mutation show distinct disturbances associated with HCM as compared to DCM. In this study, we investigated the effects of several FHC linked mutations, including well-investigated mutations such as cardiac troponin T (cTnT) I79N, R278C and F110I using a mammalian two-hybrid system. The interaction between cTnT and alpha-tropomyosin (Tm) was significantly higher for the cTnT F110I than wild-type cTnT. A DCM mutation has previously been shown to increase the affinity of cTnT for Tm. cTnT I79N and R278C mutants show similar affinity for Tm as wild-type cTnT. These results suggest that some FHC and DCM mutations may show similar changes in cTnT-Tm interactions while other mutations may not affect cTnT-Tm interactions. This work is partly supported by NIH grant HL096819.

620-Pos Board B420
A Novel Mutation in *TNNC1*-ENCODED Cardiac Troponin C Predisposes to Hypertrophic Cardiomyopathy and Recurrent Episodes of Aborted Sudden Cardiac Death
Michelle S. Parvatiyar, Andrew P. Landstrom, Jose Renato Pinto, Jingsheng Liang, Michael J. Ackerman, James D. Potter.
Mutations in *TNNC1* are still considered uncommon causes of hypertrophic cardiomyopathy (HCM) and linkage studies are not always available to determine inheritance. Here we report a clinical and functional characterization of a novel *TNNC1* mutation identified in a pediatric HCM patient. The proband hosting the *TNNC1* mutation was clinically evaluated including medical history, physical examination, 12-lead electrocardiogram, chest X-ray and genetic testing. The disease presented with multiple episodes of ventricular fibrillation and aborted sudden cardiac death. The patient demonstrated a substantial degree of hypertrophy with QT prolongation. Genetic testing indicated that the mutation was a *de novo* occurrence. Functional studies were performed as another useful tool in establishing causation of disease. cTnC-extracted cardiac skinned fibers were reconstituted with the cTnC-HCM mutation and increased the Ca^{2+} sensitivity with no effect on the maximal force. In addition, reconstituted actomyosin ATPase assays showed that the Ca^{2+} sensitivity was similar in the presence of 100% mutant cardiac Troponin C (cTnC) or 50% mutant: 50% WT cTnC. The effect of the mutation on actomyosin ATPase activation and inhibition is currently being investigated. The Ca^{2+} affinity of the cTnC mutant was evaluated by fluorescence. At all levels; troponin complex, thin filament (TF) and TF + myosin subfragment 1 (to a lesser degree) the Ca^{2+} affinity was increased. These results suggest that this mutation has a direct affect on the Ca^{2+} sensitivity of the myofilament which may alter Ca^{2+} handling and contribute to the arrhythmogenesis seen in the patient. In summary, mutations in *TNNC1* may predispose to the pathogenesis of a fatal arrhythmogenic subtype of HCM. The underlying functional alterations of the myofilament containing the mutant cTnC may further contribute to the severity of the clinical phenotype.

621-Pos Board B421
Effect of Hypertrophic Cardiomyopathy Linked Troponin C Mutations and Troponin I Phosphorylation on the Rate of Calcium Dissociation from the Thin Filament
Acchia Albury, Nicholas Swindle, Darl R. Swartz, **Svetlana Tikunova**.
The objective of this work was to investigate the effect of hypertrophic cardiomyopathy linked A8V, E134D and D145E mutations on the rate of calcium dissociation from cardiac troponin C (cTn)C on the thin filament under different phosphorylation states of cTnI. While the E134D mutation did not affect the rate of calcium dissociation, the A8V and D145E mutations led to significantly slower rates of calcium dissociation from cTnC on the thin filament. The effect of A8V, E134D and D145E mutations on the rate of calcium dissociation from the thin filament was also examined after cTnI was replaced by either PKA (S22D/S23DcTnI) or PKC (S41D/S43DcTnI) phosphorylation mimetic of cTnI. Replacement of cTnI by either the PKA or PKC phosphorylation mimetic of cTnI dramatically accelerated calcium dissociation from wild-type cTnC on the thin filament. The A8V mutation still led to significantly slower rate of calcium dissociation from cTnC on the thin filament after cTnI was replaced by the PKA phosphorylation mimetic of cTnI. However, in the presence of PKC phosphorylation mimetic of cTnI, the ability of A8V mutation to slow the rate of calcium dissociation from the cTnC on thin filament was abolished. The E134D mutation exerted minor to no effect on the rate of calcium dissociation from cTnC on the thin filament regardless of cTnI phosphorylation status. On the other hand, the D145E mutation led to significantly slower rates of calcium dissociation from cTnC on the thin filament after cTnI was replaced by either PKA or PKC phosphorylation mimetic of cTnI. Thus, the ability of hypertrophic cardiomyopathy linked cTnC mutations to affect the rate of calcium dissociation from cTnC on the thin filament varies depending on phosphorylation status of cTnI.

622-Pos Board B422
Fetal Cardiac Troponin Isoforms Rescue the Increased Ca^{2+} Sensitivity Produced by a Novel Double Deletion in Cardiac Troponin T Linked to Restrictive Cardiomyopathy
Jose Renato Pinto, Shi Wei Yang, Marc-Phillip Hitz, Michelle S. Parvatiyar, Michelle A. Jones, Jingsheng Liang, Victor Kokta, Michelle Jaeggi, Gregor Andelfinger, James D. Potter.
A novel double deletion in cardiac troponin T (cTnT) of two highly conserved amino acids (N100 and E101) was found in a restrictive cardiomyopathic (RCM) pediatric patient. Clinical evaluation revealed the presence of left atrial